Comm.
Dr. Andervont
Dr. Gardner
Dr. Sommers

ack. sent 6/4/73 gh

#915

CARCINOGENESIS

# THE COUNCIL FOR TOBACCO RESEARCH—U.S.A., INC.

110 EAST 59TH STREET
NEW YORK, N. Y. 10022
(212) 421-8885

Application for Research Grant

(Use extra pages as needed)

Date: May 29, 1973

RECEIVED
JUN 4 1973

1. Principal Investigator (give title and degrees):
Edward Bresnick, Ph.D.
Professor and Chairman

2. Institution & address:
Medical College of Georgia
Gwinnett Street
Augusta, Georgia 30902

3. Department(s) where research will be done or collaboration provided:
Cell and Molecular Biology

4. Short title of study:
Genetic and Environmental Influences on the Synthesis, Interaction with DNA, and Inactivation of the Epoxide of 3MC in Lung

5. Proposed starting date: January 1, 1974

6. Estimated time to complete: 3 years

7. Brief description of specific research aims:

a. To ascertain the extent of formation of 3-methylcholanthrene (3MC)-11,12-oxide in lung tissue obtained from a series of mice which possess varying susceptibilities to polycyclic hydrocarbon-induced tumorigenesis

b. To determine the rate of detoxification of the epoxide of 3MC in lung tissue, i.e., the activity of epoxide hydrase, obtained from these mice.

c. To ascertain the extent of the repair process in lung tissue after incubation with polycyclic hydrocarbon and the effect of 3MC and its epoxide upon the radiation-induced repair mechanism in fibroblasts.

d. To ascertain the effects of prior exposure to 3MC upon the above parameters, i.e. what effect does 3MC administration have upon epoxide synthetase, hydrase, and the repair mechanism.

e. To attempt to correlate the above parameters with polycyclic hydrocarbon-induced lung tumorigenesis.

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8. Brief statement of working hypothesis:

The remarkable spectrum of lung tumorigenesis among inbred strains of mice has suggested the involvement of genetics in susceptibility or resistance to this type of cancer. Furthermore, given a genetic susceptibility, environmental chemicals, i.e., polycyclic hydrocarbons, increase the numbers of tumors and decrease the latency time. It is the hypothesis of the present study that susceptibility may be related to a) the genetically-determined basal levels of epoxide synthetase, epoxide hydrase and the extent of the repair mechanism and b) the magnitude of the response to the prior administration of 'inducers', such as 3-MC. Those individuals who have low levels of the detoxifying enzyme, i.e., hydrase, high levels of the synthetase and a reduced repair capability would be susceptible to lung tumorigenesis. Furthermore, the response to 'inducers' should involve increased synthetase activity relative to hydrase and decreased relative repair capability. This hypothesis will be tested in mice that exhibit varying degrees of susceptibility to 3MC-induced pulmonary tumorigenesis.

9. Details of experimental design and procedures (append extra pages as necessary)

Although millions of humans are constantly exposed to an environment which is rich in polycyclic hydrocarbons through inhalation of cigarette smoke and exhaust products, only a small percentage will ultimately develop lung cancer. This observation implies the existence of populations of resistant and susceptible individuals. In the mouse, a similarly-remarkable spectrum in susceptibility to either spontaneous or polycyclic hydrocarbon-induced pulmonary tumorigenesis has been observed ranging from 100% incidence in some inbred strains to almost complete resistance.

In this regard, Andervont (1) and Shimkin (2) have reported on the comparative susceptibilities of a number of strains of mice to the induction of pulmonary tumors after administration of 1,2,5,6 dibenzanthracene and 3-methylcholanthrene (3MC), respectively. The results of these and of other studies have strongly implicated genetics as an important determinant in lung tumor development. In the studies cited above (1,2), it was also concluded that given a genetic capability, environmental factors would then play a prime role in the development of neoplastic disease. Thus, for example, the administration of 3MC to genetically-susceptible mice resulted in the appearance of more tumors and at an earlier time, i.e., shorter latent period.

It is the primary goal of this research proposal to understand the nature of the 'genetic susceptibility' and the manner in which 3MC can exert its effect. As a corollary, the mechanism of resistance of certain inbred strains of mice to polycyclic hydrocarbon-induced pulmonary tumorigenesis will be under study.

Kirschbaum Memorial Mouse Laboratory: One of the unique aspects of this proposal is the ready availability of numerous mouse model systems that have been developed over the past 30 years by Dr. A. G. Liebelt and that can now serve as the source material for the objectives set forth in this proposal.

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These mouse model systems were developed in the Kirschbaum Memorial Laboratory, a facility which was established at Baylor College of Medicine in 1958 following the death of the late Dr. Arthur Kirschbaum and which has been recently moved (in 1971) to the Medical College of Georgia. The mouse colony is still under the direction of Dr. Annabel Liebelt and consists of 17 different inbred strains of mice that have been maintained by brother-sister matings since their introduction into the colony, in some cases over 40 years ago. All strains are tested periodically for genetic homogeneity by reciprocal skin grafting techniques. In addition, all autopsies of all experimental and colony mice have been carried out either personally or under the direct supervision of either Dr. A. G. Liebelt or Dr. R. A. Liebelt for the past 15 years thereby assuring a uniform pattern of data collection. A computer program written in PLI language was initially developed at Baylor College of Medicine and is being continued here at the Medical College of Georgia to facilitate retrieval and analyses of available data now kept in record books.

The available mouse strains include:

Strain: A/Ki
Origin: Strong to Kirschbaum in 1944
Charac: mammary cancer incidence 80% in breeding ♀♀, 30% in virgins - enhancement after methylcholanthrene. Reticular tissue neoplasms, primarily histiocytomas, 4% in breeding ♀♀, 12% in ♂♂. Reciprocal skin grafts with Af/BiKi not accepted.
Inbreeding: F? + 66 (66 in this laboratory)

Strain: Af/BiKi
Origin: Bittner (who called it Ax) to Liebelt in 1961
Charac: mammary cancer less than 1% in breeding ♀♀ and leukemia less than 1%. Reciprocal skin grafts with A/Ki not accepted
Inbreeding: F61 by Bi after fostering and F24 in this laboratory

Strain: AKR/Ki
Origin: NIH to Ki in 1956
Charac: leukemia primarily lymphatic and involving thymus - 83% in ♀♀ at av. age 9 1/2 mo., 68% in ♂♂ at av. age 10 1/2 mo.; ♂♂ at av. age 10 1/2 mo., ♂♂ fight.
Inbreeding: F46 in this laboratory

Strain: BALB/cKi
Origin: Bittner to Kirschbaum 1952
Charac: mammary cancer less than 8% in breeding ♀♀ (primarily in one subline) - enhanced after methylcholanthrene - enhanced after MTA introduced. Leukemia less than 12% in ♀♀ (primarily in one subline) - enhanced after methylcholanthrene - enhanced after MTA introduced. Leukemia less than 12% in ♀♀ and less than 10% in ♂♂.
Reciprocal skin grafts with BALB/cKi accepted.
Inbreeding: F65 by Bittner and F31 in this laboratory.

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Strain: CBA/Ki
Origin: Strong (probably) - Kirschbaum 1941
Charac: mammary cancer less than 2% in breeding ♀♀. Leukemia less than 6% in ♀♀, less than 3% in ♂♂. Hepatomas less than 2% ♀♀, 9% ♂♂ at approx 1 1/2 years. Uniformly susceptible to 0.35 mg/gm BW goldthioglucose - 100% survival and obesity. Reciprocal skin grafts with CBA/StKi not accepted. Has retinal degeneration
Inbreeding: F? + 61

Strain: CBA/StKi
Origin: Strong to Liebelt 1963
Charac: mammary cancer 40% in breeding ♀♀. Reciprocal skin grafts with CBA/Ki not accepted. Does not have retinal degeneration
Inbreeding: F? + 37

Strain: CE/Ki
Origin: (Wooley?) to Kirschbaum 1952
Charac: mammary cancer 3% in breeding ♀♀, ovarian tumors less than 10%, reticular tissue neoplasms less than 3%
Inbreeding: F? + 37

Strain: C3H/BiKi
Origin: Bittner (Z) to Kirschbaum 1972
Charac: mammary cancer 83% in breeders, variable in virgins. Leukemia less than 0.5% in breeding ♀♀, 14% in ♂♂. Hepatomas 0% in ♀♀, 10% in ♂♂. Reciprocal skin grafts with C3H/BifB/Ki accepted
Inbreeding: F80 by Bittner and 41 in this laboratory

Strain: C3H/BifB/Ki
Origin: Bittner's Zb - C3H fostered on C57Bl. Bi to Kirschbaum 1956
Charac: mammary cancer 2% in breeding ♀♀. Leukemia 9% in ♀♀, 3% ♂♂. Hepatomas 2.5% ♀♀, 12% ♂♂. Reciprocal skin grafts with C3H/BiKi accepted. Grafts with C3Hf/Crgl not accepted
Inbreeding: F117 (83 by Bi, 34 by Ki)

Strain: C57BL/Ki
Origin: Bittner to Kirschbaum 1951
Charac: mammary cancer 0% in breeding ♀♀. Reticular tissue neoplasms less than 10% in ♀♀, less than 4% in ♂♂. Some hydrocephalus
Inbreeding: F? + 45

Strain: DBA/2Ki
Origin: Bittner to Kirschbaum 1944
Charac: mammary cancer 66% in breeding females, 30% virgins. Leukemia 6% ♀♀, 8% ♂♂. Both types of cancer enhanced with methylcholanthrene.
Inbreeding: F? + 56

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Strain: DBA/$2_f$Ki
Origin: Bittner fostered DBA/2 on Zb in 1945 - to Kirschbaum
Charac: mammary cancer less than 3% in breeders; leukemia 10% ♀♀, 2% ♂♂
Inbreeding: F? + 49

Strain: DBA/$2_{ff}$Ki
Origin: Kirschbaum fostered DBA/2f (low cancer) on C3H (+) in 1945
Charac: mammary cancer 76% in breeding females, 43% in virgins. Leukemia 5% females, 4% males
Inbreeding: F? + 51

Strain: FB/Ki
Origin: Kirschbaum 1942 - [ (A x F) X F] x [ (A x F) X F] - FA
1952 - [ (NH x FA) x FA] x FA = FB
Charac: reticular tissue neoplasms approx 50%; some myeloid leukemia
Inbreeding: F36

Strain: $I^s$/BiKi
Genet.: no chinchilla as $I^b$ and I
Origin: Strong 1950 to Bittner - to Liebelt 1961
Charac: resistant to mammary cancer - do not propagate MTA. Transmit inducing hormonal pattern for mammary cancer
Inbreeding: F74 by Bittner and F19 in this laboratory

Strain: NH/Ki
Origin: Kirschbaum 1942
Charac: Low tumor incidence; nodular hyperplasia and adrenal adenomas in at least 10% of old mice--gonadectomy at 6 weeks enhances adenomas. Breeding ceases at approx. 9 months. Some obesity. One subline losing piebald and becoming albino
Inbreeding: F70

Strain: YBR/Ki
Genet: $\underline{A^y a}$
Origin: Heston to Liebelt 1958
Charac: amyloid at least 50% in males and females with $A^y a$ or aa. Obesity in F1 hybrids between $A^y a$ and other strains. Low tumor.
Inbreeding: F65 (He) + 29 (Ki)

These straims include those which are highly susceptible to pulmonary tumors, Balb/c, NH, A, CBA; moderate to low incidence, DBA, C3H, FB, $I^s$, YBR; and low to no incidence, C57Bl, AKR.

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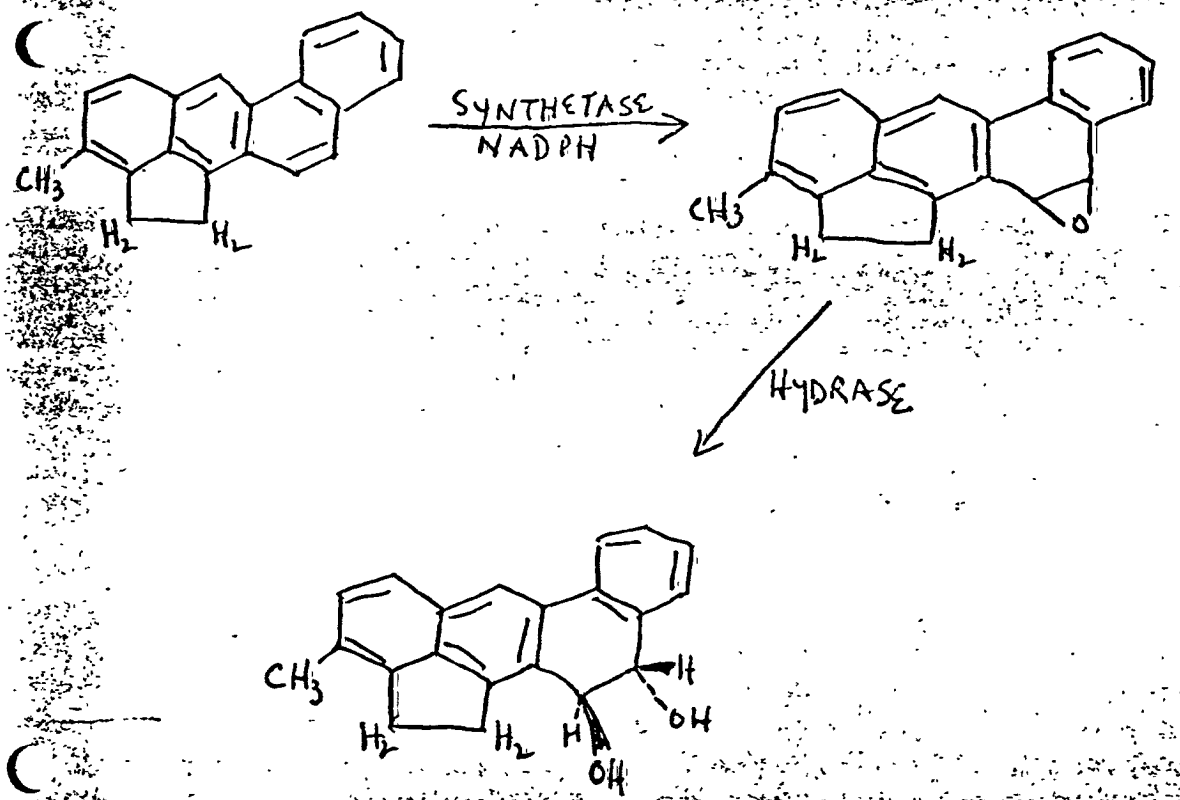
Aryl Hydrocarbon Hydroxylase and 3MC Metabolism: The administration of 3MC to rodents or its addition to in vitro systems is accompanied by the appearance of a number of metabolites including the 1 (or 2) hydroxy, 1 (or 2) keto, cis and trans 1,2 dihydroxy, cis and trans 11,12 dihydro 11,12 dihydroxy, and 11,12 epoxy derivatives (3-6). The biotransformation of 3MC to the metabolites is catalyzed by the microsomal NADPH-dependent enzyme, aryl hydrocarbon hydroxylase. The latter enzyme system is present and "inducible" in vivo by polycyclic hydrocarbons and other substances in a number of tissues, including lung. However, the basal and "inducible" levels of aryl hydrocarbon hydroxylase vary greatly among the various tissues, different species and various strains of a mammalian species; genetic factors apparently influence the level of control enzyme as well as the extent of the response (induction) to environmental influences, i.e., polycyclic hydrocarbons (4,7).

Aryl hydrocarbon hydroxylase is a complex system which catalyzes diol formation presumably through a reactive intermediate, an epoxide. The sequence of reactions leading to the formation of the trans 11,12 dihydro-11,12-dihydroxy 3MC from 3MC is indicated below. The product of the 1st reaction is the 11,12-oxide catalyzed by epoxide synthetase; diol formation proceeds through the catalysis of epoxide hydrase.

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It was Boyland (8) who initially proposed in 1950 that epoxides may be formed from aromatic ring structures as intermediates in the transition to diols and phenols. Since that time, epoxides have been reported as intermediates in the metabolism of polycyclic hydrocarbons (9-11). The formation of epoxides (particularly at the 11,12 double bond or K-region) has great import in view of the production of mutational changes i.e., "malignant" transformation, upon addition to cultured mammalian cells (12). The trans diol, on the other hand, would represent a less carcinogenic substance. Consequently, not only is the rate of synthesis of the K-region epoxide of 3MC important, but the subsequent rate of hydration must be given prime consideration. The intracellular quantity of the reactive epoxide will therefore be a function of the levels of epoxide synthetase and hydrase enzymes.

As noted previously, aryl hydrocarbon hydroxylase activity is markedly induced in tissues by prior administration of 3MC. It is not clear, however, whether this induction is the result of increased activity of the epoxide synthetase, hydrase, or both these components.

Assay for 3MC Epoxide Hydrase Activity in Lung: Because of the importance of this enzyme in the inactivation of 3MC, we propose to assay 3MC epoxide hydrase activity in microsomal preparations of lungs obtained from mouse strains which are susceptible to 3MC-induced pulmonary neoplasms, i.e., Balb/c, NH, A, CBA; from mouse strains which are only moderately susceptible, i.e., DBA, $C_3H$. FB, $I^S$, YBR; and from those which are resistant to carcinogenesis, i.e., $C_{57}Bl$, AKR.

The basic assay for epoxide hydrase depends upon separation of the trimethylsilyl (TMS) derivative of the trans diol from that of the 11,12 oxide (the latter is decomposed to the 11 or 12 TMS derivative). Since the method is in press (13,14) and has not appeared yet, it will be described in some detail. Furthermore, preprints are included in the Appendix.

The 11,12 oxide as well as the cis and trans diol derivatives of 3MC were prepared by the method of Sims (5). The trimethylsilyl derivatives were formed according to the following procedure: To 1 mg of compound was added 0.4 ml of Tri-Syl$^R$ (Pierce Chemical Company) and after the resultant mixture was heated at 60° for 30 min, 0.2 ml of N,O-bis-TMS-trifluoroacetamide (Pierce Chemical Company) was introduced. The solution was brought to a final concentration of 1 mg polycyclic hydrocarbon per ml by the addition of acetonitrile and stored overnight at 5°. The derivatives were stable under these conditions for 2 days. All steps were conducted under conditions of reduced light.

The gas chromatography was conducted in a Nuclear Chicago Model 5000 instrument containing a U-tube glass column, 4 mm x 6 ft, in which was packed 100-120 mesh gas chrom Q coated with 3% OV-17 (Applied Science

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Laboratories). The temperature was programmed from 220°-320° at 3°/min. The mobilities of the substances were expressed as methylene units in comparison to the retention of known hydrocarbon standards (Table I). The identities of the TMS derivatives were established by mass spectrometry.

TABLE I

Retention of 3MC Derivatives on OV-17

| TMS Derivative of | Methylene Units |
|---|---|
| cis 11,12 dihydro 11,12 dihydroxy 3MC | 32.54 |
| trans 11,12 dihydro 11,12 dihydroxy 3MC | 29.63 |
| 11,12 oxide | 36.00 |

Although the hydration of styrene oxide has been employed as the basis for the hydrase assay in previous work (15), this reaction might not reflect an accurate assay of the formation of trans dihydrodiol derivatives of polycyclic hydrocarbons. The assay just described is specific for 3MC. The gas chromatographic assay also has the following advantages: 1) it employs relatively simple substrates and can be employed for all aromatic epoxides. 2) difficulties associated with radioactive contamination, tritium exchange reactions, and differential isotope effect are avoided. 3) the assay is extremely sensitive and can detect less than 0.2 nmoles of diol.

Following incubation of 3MC-11,12 oxide with the tissue preparation, the products are extracted into ethyl acetate. The latter was evaporated and the TMS derivatives were prepared.

The major enzyme activity in liver is associated with the microsomal fraction, although the 600-20,000 x g pellet fraction possessed a specific activity which was approximately 30% of that seen with the 100,000 x g pellet (4.0 vs11-12 nmoles diol formed per mg protein per 10 min.). A good deal of the former activity may be associated with microsomal contamination. No activity could be found in the supernatant fraction.

The reaction with liver appeared linear for up to 10 min and consequently, the latter time was chosen as a part of the assay conditions. A study of velocity vs substrate concentration of the reaction indicated that 0.44 mM epoxide (50 µg) was sufficient to saturate the system; a calculated Km for this substrate was $2.8 \times 10^{-4}$ M. Enzyme activity was also directly proportional to the protein concentration of liver microsomes, i.e., to 10 mg protein.

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Liver microsomes possessed the greatest amount of activity whereas microsomes obtained from kidney and lung appeared to be 25% and 5% as active, respectively. No demonstrable enzyme activity could be detected in microsomes obtained from either spleen or small intestine. Finally, as reported by Oesch and colleagues (16) for the hydration of styrene oxide, equimolar quantities of 1,1,1-trichloropropene oxide (TCPO) completely inhibited the formation of the diol from 3MC-epoxide.

This series of experiments will allow us to examine the basal levels of 3MC epoxide hydrase in lung microsomes from the various mouse strains.

3MC Epoxide Synthetase Activity: This enzyme is a key in the activation of 3MC to the 11,12 oxide. Although in previous studies, its activity was semi-quantitatively measured by employing labeled 3MC in the presence of a bank of epoxide, we propose to modify this assay as follows:

Epoxide synthetase can be measured by employing the same assay conditions as described for the hydrase but with 3MC as substrate and with the inclusion of NADPH-generating system. In addition, an inhibitor of hydrase, TCPO, will be added to block the subsequent hydration of the epoxide. Under the conditions of the assay, 3MC is not derivatized to any TMS compound and the 11,12 oxide is converted to the 11 or 12 TMS derivative.

In addition, we intend to modify the assay conditions for synthetase activity so as to achieve greater sensitivity by employing the electron capture detector. Toward this end, 3MC will be incubated with lung microsomes, TCPO and a NADPH-generating system. Appropriate controls, i.e., heated or no microsomes, as well as no NADPH, will run simultaneously. The metabolic products will be extracted as described in the hydrase section and derivatized with perfluorobenzoylchloride. Under these conditions the 11,12 oxide is converted to the 11 or 12 phenol which is coupled to form the perfluorobenzoate ester. The latter can be determined with great sensitivity by gas chromatographic techniques with the electron capture detector. It may be necessary to 'clean' up the derivative first by thin layer chromatography on silica gel with benzene as the developing solvent. These studies are presently underway.

After establishing the validity of this synthetase assay, we will then be able to catalog the 3MC epoxide synthetase activity in lung microsomes obtained from the various mouse strains.

Induction of microsomal enzyme activity: Having established the basal levels of the synthetase and hydrase in lung tissue obtained from susceptible, moderately-susceptible and resistant mice (to polycyclic hydrocarbon carcinogenesis), we will then examine the response

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of these enzymes to 3MC administration. The latter will be injected in corn oil i.p., into male mice, 6-8 weeks of age, at a level of 20-100 mg/kg body wt and the synthetase and hydrase activities will be ascertained in lung microsomes as described above, at various times after administration of the polycyclic hydrocarbon. Suitable control mice of the same age and sex will be injected with the vehicle alone and enzyme activities determined. In this manner, we will be able to determine if prior exposure to 3MC can influence the levels of either the synthetase, hydrase or both in lung tissue obtained from the various mouse strains.

In addition to 3MC, we propose also to examine the effects of other 'inducers', e.g., β-naphthoflavone (370 μmoles/kg i.p. in corn oil), phenobarbital (75 mg/kg, twice daily for 3 days, i.p.), and pregnenolone 16α carbonitrile (10 mg/kg, 5 times at 12 hr intervals, i.p.) upon synthetase and hydrase activities in lung tissue at appropriate intervals after drug treatment.

Extent of the Repair Mechanism in Lung: Mammalian cells have a remarkable capacity to repair by "unscheduled DNA synthesis" its genetic material which might have been damaged by a variety of agents including UV irradiation, carcinogenic and non-carcinogenic alkylating agents. Consequently, this repair mechanism represents a fundamental life process which may have important implications in chemical carcinogenesis. In fact, a reduced level of DNA repair synthesis has been noted in cells from patients with Xeroderma pigmentosum, a condition manifested by increased sensitivity to UV irradiation and increased skin cancer (17). Since it has been postulated that all carcinogens interact covalently with macromolecules, including DNA (18), the extent to which a tissue can repair this 'hit' may in part determine its susceptibility to chemically-induced tumorigenesis. We propose to study the repair process initially in fetal lung explants which have been subjected to 3MC or its 11,12 oxide under culture conditions. The latter will be similar to those employed with fetal liver as reported previously (3,4); we have shown that lung can be used under these conditions. The explants will be preincubated with 3MC or the 11,12 oxide for varying periods of time and then incubated with $^{3}$H-thymidine in the presence of hydroxyurea ($10^{-2}$ M). Under these conditions, S-phase DNA synthesis is completely abolished and only unscheduled repair continues. The relative rates of $^{3}$H-thymidine incorporation into DNA will be determined by standard methods, as have been used in this laboratory, in the presence and absence of hydroxyurea after preincubation with or without 3MC or its oxide. Additional purification of the isolated DNA may be required by isopycnic centrifugation in CsCl as outlined by Flamm (19). From these results, we should be able to obtain a first approximation as to the extent of the repair mechanism in lung tissue from various mouse strains.

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The objective of these studies is to determine the extent of the repair system after addition of 3MC and its 11,12 oxide to fetal lung preparations obtained from various mouse strains.

The extent of binding to lung DNA of $^{3}H$-labeled 3MC, which had been injected subcutaneously into male mice of different strains, will also be ascertained at various times after administration of the polycyclic hydrocarbons. We should be able to obtain some information about the rate of binding under these in vivo conditions as well as the rate of disappearance of the label from the lung DNA over the course of time.

It will be of importance to determine a) the effects of prior administration of 'inducers' e.g., β-naphthoflavone, upon the in vivo binding of labeled 3MC to lung DNA and the disappearance of label from the latter b) the effects of addition of inducers upon the level of "unscheduled DNA synthesis" in the fetal lung explant system.

Effect of 3MC and the 11,12 Oxide on the Repair Mechanism: It is possible that some of the action of 3MC (or its metabolites) may be due to interference with the repair mechanism. Accordingly, we propose to use the fibroblast system for this phase of the investigation. Human fibroblasts (originally obtained from a prepuce) are routinely grown in culture in this Department. We propose to investigate the "unscheduled synthesis of DNA" after UV irradiation of these cells (10 erg/mim$^2$/sec by 15 watt germicidal lamp) followed by their incubation with $^{3}H$-thymidine in the presence of 10 mM hydroxyurea, largely as outlined by Brandt et al (20). After establishing the baseline levels for repair with these cells 3MC or its 11,12 oxide will be added during the repair phase and the extent of incorporation will be determined.

The research outlined in this proposal represents approximately 3 years effort. The first priority will be given to establishing the base line levels of synthetase and hydrase in lung microsomes obtained from various mouse strains. These studies will be followed by the induction experiments, i.e., after administration of 3MC or β-naphthoflavone to the mice, and finally, the series on repair will be conducted later.

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10. Space and facilities available (when elsewhere than item 2 indicates, state location):

The principal investigator has approximately 1500 sq ft of laboratory space in the Sanders Research and Education Building in addition to access to cold rooms, culture rooms (with lamilar flow hoods) and the mouse colony (Kirschbaum Memorial Mouse Laboratory). The major items of equipment presently in the laboratory include: liquid scintillation spectrometers; microscopes; water-jacketed $CO_2$ incubators; all types of centrifuges; electrophoretic and chromatographic equipment; spectrophotofluorometers and fluorimeters; spectrophotometers; Nuclear gas chromatograph with FID and Beckman GC-65 with electron capture. In addition, we have access to a Zeiss electron microscope. In short, we have all the necessary equipment and space to accomplish the aims of this research proposal.

11. Additional facilities required:

No additional facilities required.

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12. Biographical sketches of investigator(s) and other professional personnel (append):

See Enclosure

13. Publications: (five most recent and pertinent of investigator(s); append list, and provide reprints if available).

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R: REDACTED MATERIAL



14. First year budget:

| A. Salaries (give names or state "to be recruited") Professional (give % time of investigator(s) even if no salary requested) | % time | Amount |
|---|---|---|
| Edward Bresnick, Prin. Invest. | 25 | REDACTED |
| Terry A. Stoming, Ph.D. | 100 | |
| Annabel G. Liebelt, Ph.D. | 10 | - |
| Technical Research Assistant II | 100 | REDACTED |
| | | REDACTED |
| Fringe Benefits | | |
| Sub-Total for A | | REDACTED |

| B. Consumable supplies (by major categories) | |
|---|---|
| Chemicals | 2,000 |
| Radioisotopes | 2,000 |
| Laboratory glassware | 2,000 |
| Sub-Total for B | 6,000 |

| C. Other expenses (itemize) | |
|---|---|
| Boarding charges for mice | 3,000 |
| Travel for 2 to 1 national meeting | 800 |
| Maintenance contracts (for centrifuges, etc.) | 500 |
| Sub-Total for C | 4,300 |
| Running Total of A + B + C | 35,348 |

D. Permanent equipment (itemize)
None

ok
wm

| | |
|---|---|
| Sub Total for D | - |
| E | 5,302 |
| Total request | 40,650 |

E. Indirect costs (15% of A+B+C)

15. Estimated future requirements:

| | Salaries | Consumable Suppl | Other Expenses | Permanent Equip | Indirect Costs | Total |
|---|---|---|---|---|---|---|
| Year 2 | REDACTED | 6,000 | 4,300 | - | 5,478 | 41,997 |
| Year 3 | | 6,000 | 4,300 | - | 5,714 | 43,806 |

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16. Other sources of financial support

List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

| Title of Project | Source (give grant numbers) | Amount | Inclusive Dates |
|---|---|---|---|
| None at this time | | | |

PENDING OR PLANNED

| Title of Project | Source (give grant numbers) | Amount | Inclusive Dates |
|---|---|---|---|
| Not at this time | | | |

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal Investigator

Typed Name Edward Bresnick, Ph.D.

Signature Edward Bresnick Date 5/30/73

Telephone 404-724-7101 Ext. 8721
(Area Code / Number / Extension)

Checks payable to

James A. Blissit, Vice President/Treasurer

Mailing address for checks

Medical College of Georgia

Augusta, Ga. 30902

Responsible officer of institution

Typed Name Robert A. Liebelt, M.D., Ph.D.

Title Provost

Signature Robert A. Liebelt Date 6/1/73

Telephone 404-724-7111 Ext. 8863
(Area Code / Number / Extension)

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R: REDACTED MATERIAL

6

CURRICULUM VITAE

EDWARD BRESNICK
REDACTED

Social Security Number:

Date and Place of Birth

Sex: Male

Marital Status: REDACTED
REDACTED

Education: St. Peter's College, B.S., magna cum laude,
Fordham University, M.S., REDACTED
Fordham University, Ph.D.,

Positions: Research Associate, University of Texas Medical Branch, 1957-1958
Research Biochemist, Wellcome Research Labs., 1958-1961
Senior Research Biochemist, above, 1959-1961
Assistant Professor of Biochemistry, Baylor University College of Medicine, 1961-1963
Assistant Professor of Pharmacology, Baylor University College of Medicine, 1963-1965
Associate Professor of Pharmacology, Baylor University College of Medicine, 1965-1968
Professor of Pharmacology, Baylor University College of Medicine, 1968-1971
Professor and Chairman, Department of Cell and Molecular Biology, Medical College of Georgia, 1971-present
Acting Dean, School of Graduate Studies, Medical College of Georgia, 1972-present

Scientific Societies:

REDACTED

Honorary Societies:

Phi Lambda Upsilon
Sigma Xi

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Awards: Lederle Medical Faculty Award, 1966-1969

National Committees:

Member of the Pharmacology-Toxicology Review Committee for the National Institute of General Medical Science, 1968-1972.

Other Honors:

Award for "Outstanding Basic Science Faculty Member," 1966.
Award for Excellence in Teaching, by Phi Chi Medical Fraternity, 1968
President, Texas Medical Center Research Society, 1968
Elected to Represent Basic Sciences on the Executive Faculty, 1969

Functions in the University:

Medical School (Baylor):

Elected Representative on the Executive Faculty, 1969
Member, Medical Admissions Committee
Chairman, Subcommittee for Minority Admissions
Member, Radioisotope Committee
Member, Faculty Research and Fellowships Support Committee
Member, Curriculum Committee
Vice-chairman, Student Affairs Committee
Member, Subcommittee of Faculty Advisors

Graduate School (Baylor):

Member, Graduate Executive Committee
Chairman, Cell Biology Committee
Member, Graduate Admissions Committee
Member, Course and Curriculum Committee
Member, Scholarships Committee
Director of Graduate Studies, Department of Pharmacology, 1966-1968

Medical School (Medical College of Georgia):

Member, Senior Administrative Group
Member, Executive Committee
Member, Phase I-II Curriculum Committee
Member, Long Range Planning Committee
Member, Physiology Search Committee
Member, Pathology Search Committee
Member, Grants and Contracts Advisory Committee
Member, Board of Directors of the Augusta Radiation Therapy Center, Augusta, Georgia
Member, Professional Services Committee of the Augusta Radiation Center and Tumor Institute, Inc.
Member, Administrative Council
Member, Health Communications Advisory Committee
Member, Academic Council of the School of Nursing
Elected Faculty Representative to the Student Council (1973) of the Medical College of Georgia.

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Graduate School (Medical College of Georgia:

Member, Graduate Council Committee

Editorial Work: Journal of Pharmacology and Experimental Therapeutics, Field Editor.

Biochemical Pharmacology, Editor.
Member, Board of Honorary Editorial Advisers of Biochemical Pharmacology

Mentioned in:

American Men of Science
World Who's Who in Science
Who's Who in America
Dictionary of International Biography
Community Leaders of America

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Source: https://www.industrydocuments.ucsf.edu/docs/khpl0000

Publications:


1. Yee, M. and Bresnick, E.: Effects of administration of 3-methylcholanthrene upon the 2M NaCl-extractable chromatin proteins in rat liver. Mol. Pharm., 7:191-198, 1971.

2. Bürki, K., Seibert, R. A., and Bresnick, E.: Induction of Benzpyrene Hydroxylase Activity in Fetal Rat Liver Explants: Metabolism of 3-Methylcholanthrene and Differential Effects of Its Derivatives of Benzpyrene Hydroxylase Activity Biochimica et Biophysica Acta, Vol. 260, pp. 98-109 (1972).

3. Bürki, K. and Bresnick, E. : Cellular Responses to Methylcholanthrene: The Role of Benzpyrene Hydroxylase in the Binding of Polycyclic Hydrocarbon to Cytosol Macromolecules in Fetal Rat Liver Explants. Arch. Biochem. & Biophys., Vol. 152, pp. 574-589, 1972.

4. Bürki, K., Liebelt, A. G. and Bresnick, E.: Induction of Aryl Hydrocarbon Hydroxylase in Mouse Tissues from a High and Low Cancer Strain and their $F_1$ Hybrids. J. Nat'l Cancer Inst. Vol. 50, pp. 369-380, 1973.

5. Stoming, T. A, Knapp, D. R., and Bresnick, E.: Gas Chromatographic Separation of the K-Region Epoxide and of the Cis and Trans 11,12 Diol Derivatives of 3-Methylcholanthrene. Life Sci. Vol. 12, Part II, pp.425-429, 1973.

6. Bresnick, Edward, Stoming, T. A., A Gas Chromatographic Assay for Epoxide Hydrase Activity with 3-Methylcholanthrene-11,12-Oxide Science, accepted.


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R: REDACTED MATERIAL



Terrance A. Stoming
Department of Cell and Molecular Biology
Medical College of Georgia
Augusta, Ga. 30902
Telephone: 724-7111 (Ext. 8744)

Personal: REDACTED

Professional Objective: Teaching and conducting research in the area of medicinal and biochemical organic chemistry at the university level.

Education: REDACTED, Purdue University
Major: Pharmacal Sciences
Grade index: 5.10/6.00

REDACTED
Purdue University
Major: Synthetic Medicinal Chemistry

Ph.D., January R Purdue University
Major: Synthetic Medicinal Chemistry
Grade index: 5.43/6.00

Experience

November, 1971 - present: Medical College of Georgia
Department of Cell and Molecular Biology
Post-doctoral research
Augusta, Georgia

1969 to November, 1971: Purdue University
School of Pharmacy and Pharmacal Sciences
Department of Medicinal Chemistry
Lafayette, Indiana 47907
Graduate Research. The design and synthesis of potential histamine agonists.

1966 to 1969: Purdue University
School of Pharmacy and Pharmacal Sciences
Department of Medicinal Chemistry
Lafayette, Indiana 47907
Graduate Research. The Attempted Synthesis of a Possible Cyclopentanoterpenoid Precursor.

1965 to 1966: Purdue University
School of Pharmacy and Pharmacal Sciences
Department of Medicinal Chemistry
Lafayette, Indiana 47907
Undergraduate research. The Synthesis of Ring Substituted α-isopropylaminophenones.

Fellowships

1970 to 1971 American Foundation for Pharmaceutical Education., E. Mead Johnson Memorial Fellow. Teaching Assistant, Biochemistry.

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Fellowships
1969 to 1970: American Foundation for Pharmaceutical Education
Teaching Assistant, Medicinal Chemistry

1966 to 1969: N.D.E.A. Title IV Research Fellow

1965 to 1966: N.S.F. Undergraduate Research Fellow

Military Service: None
Present Draft Status: 3-A

Professional and Honor Societies: REDACTED

Publications: The Design and Synthesis of Potential Histamine Agonists (Awaiting Pharmacological Evaluation) will consist of two (2) publications.

Gas Chromatographic Separation of the K-region epoxide and of the Cis- and Trans-11,12-Diol Derivatives of 3-Methylcholanthrene. Life Sciences II 12, 425 (1973).

A Gas Chromatographic Assay for Epoxide Hydrase Activity with 3-Methylcholanthrene-11,12-Oxide Science, in press.

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Source: https://www.industrydocuments.ucsf.edu/docs/khpl0000

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12

CURRICULUM VITAE

ANNABEL GLOCKLER LIEBELT, B.A., M.S., Ph. D.

Date and place of birth: REDACTED

Children:

Social Security Number:
REDACTED

REDACTED

Education:

Public schools in Washington, D. C. - Calvin Coolidge High, REDACTED
Western Maryland College, Westminster, Maryland, R
Major: Biology. Minors: English, Philosophy
University of Illinois College of Medicine, Chicago, Illinois
R Major: Anatomy, Advisor: Dr. Arthur Kirschbaum
Thesis: Homoplastic Skin Grafting in Inbred Mice R
Baylor University College of Medicine, Houston, Texas,
Ph. D. Major: Anatomy, Minor: Microbiology. Advisors:
Dr. Arthur Kirschbaum (deceased May 28, 1959) and
Dr. John J. Trentin. Thesis: Transplantation of Normal and Neoplastic Tissues in Inbred Mice Differing at "Weak" Histocompatibility Loci.

Positions:

Biologist - National Cancer Institute, Department of Pathology, Bethesda, Maryland, October 1949 to October 1952. Dr. Harold Stewart.
Teaching and Research Assistant, Department of Anatomy, University of Illinois College of Medicine, Chicago, Illinois. Also supervisor of mouse colony and experimental projects, November 1952-1954, Dr. Arthur Kirschbaum.
Teaching and Research Assistant, Department of Anatomy, Baylor University College of Medicine, Houston, Texas. Also supervisor of mouse colony and research projects, August 1954 to 1958.
Instructor in Anatomy, Baylor University College of Medicine, 1958-1962
Assistant Professor, Baylor University College of Medicine, 1962-1967.
Associate Professor, Baylor University College of Medicine, 1967-1971.
Associate Professor, Department of Cell and Molecular Biology, Medical College of Georgia, Augusta, Georgia, 1971 - present.

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Director of Kirschbaum Memorial Laboratory (Mouse Colony with maintenance of 17 inbred strains of mice used for research and teaching).

Societies:

REDACTED

Honors and Awards:

Invited Speaker - Carcinogenesis, A Broad Critique, March 1966. Twentieth Annual Symposium in Fundamental Cancer Research, M.D. Anderson Hospital and Tumor Institute

Research Interests:

1. Factors involved in transplantation of normal and neoplastic tissues - genetic, hormonal, immunologic.

2. Host-tumor interrelationships in mice with spontaneous, induced, and transplanted tumors - hormones, plasma proteins and other blood paramenters, extramedullary hematopoiesis, viruses, development of other disease, and aging,

3. Genetic, viral and hormonal aspects involved in normal development, preneoplasia and neoplasia of the mouse mammary gland - induction and therapy of tumors.

4. Etiologic factors in spontaneous and induced leukemias in inbred mice and hybrids - genetic, hormonal, viral, chemical and physical.

5. Neuroendocrine mechanisms and neoplasia - cancer of endocrine organs and their target tissues - and relationship to obesity, hypothalamic lesions, ablation of organs, administration of exogenous hormones.

6. Biology and cytology (histochemistry and electron microscopy) of various mouse neoplasms, including mammary, ovarian, adrenal, pituitary, and liver tumors, melanomas, and neoplasms of the reticular tissue. Certain biochemical parameters and their correlation to morphological alterations.

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